population of altered light chain variable region encoding nucleic acids is constructed, wherein each altered light chain variable region encoding nucleic acid of said population of altered light chain variable region encoding nucleic acids encodes for a polypeptide, said polypeptide comprising i) a different amino acid at one or more positions when compared to said corresponding acceptor framework regions of said second reference sequence, and ii) a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region of said first reference sequence.

68. (Amended) The method of Claim 67, wherein said visual representations of first and second reference sequences are in electronic form.

R E M A R K S

Applicants appreciatively acknowledge the Examiner's withdrawal of (a) the rejection of claims 42 and 51 under 112, first paragraph, (b) the rejection of claims 42-51 under 112, second paragraph, and (c) the rejection of claims 42-51 under 103(a) in light of Deng *et al.* (Canadian patent application 2,125,240 A1). Claims 42-71 are pending. The Examiner has offered the following new grounds of rejection:

1. Claims 42-71 stand rejected under 112, second paragraph as allegedly unclear with respect to the meaning of the phrase "providing a representation."

2. Claims 42-71 stand rejected under 112, second paragraph as allegedly providing insufficient antecedent basis for the limitations "said acceptor framework region reference sequences", "said framework positions" and "said acceptor framework positions".

3. Claims 42-71 stand rejected under 103(a) as allegedly obvious in light of Deng *et al.* (Canadian Patent Application 2,125,240 A1) and further in view of Yelton *et al.* (The Journal of Immunology 155:1994-2004 [1995]) and Hagiwara *et al.* (US Patent No. 5,589,573, issued Dec. 31, 1996).

1. The Claims Are Clear and Definite

Claims 42-71 stand rejected under 112, second paragraph as allegedly unclear with respect to the meaning of the phrase "providing a representation." Applicants disagree. Speaking generally (and not with regard to the specific invention), the art recognizes that one can work with the physical nucleic acid - or one might work with a representation of the physical nucleic acid. Speaking now specifically about the present application, Figure 1 is a representation of sequences. Figure 1 is a representation, in that it provides art-recognized symbols[1] for the sequence. Moreover, Figure 1 is an alignment such as might be used in a manner taught in the claims (e.g. "wherein said framework positions that are changed are selected from among said acceptor framework positions of said second reference sequence that differ . . ") because alignments facilitate such comparisons to determine differences. Figure 1 is discussed in Example 1 (see page 51 of the specification) in a manner that makes it clear to the reader that Figure 1 is a representation of sequences. In the case of certain embodiments of the present invention, there are advantages to working with representations of nucleic acid rather than the physical nucleic acid.

Moreover, in Example 1, overlapping oligos were synthesized to construct/encode VH and VL of murine anti-CD40, "*based on the sequence* of anti-CD40 murine mAb 40.2.220." (emphasis added). From this, it is clear to one skilled in the art that a representation (whether hard copy or electronic copy) of the referenced sequence was used to design the overlapping oligos.

While the term "representation" is clear, applicants have amended the claims to further clarify that the representation is (like Figure 1) a "visual" representation, i.e. symbols that are visible to the eye (whether on a computer screen or on a piece of paper). This amendment is made to further the prosecution and applicants hereby reserve the right to prosecute the unamended claims in the future.

2. There is Proper Antecedent Basis

Claims 42-71 stand rejected under 112, second paragraph as allegedly providing

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[1] Webster's College Dictionary (Random House 1991) notes that a representation is an "expression or designation [using a] . . . character, symbol, or the like." Thus, a representation is more than a "thought pattern."

insufficient antecedent basis for the limitations "said acceptor framework region reference sequences", "said framework positions" and "said acceptor framework positions". Applicants do not agree and believe that the claims are clear as previously submitted. Nonetheless, to further the prosecution, and without waiving the right to prosecute the unamended claims in the future, the claims have been amended to make clear the distinction between "donor" and "acceptor" sequences.

3. The Claimed Embodiment Is Not Obvious

The Examiner alleges that the claims are obvious and unpatentable over Deng *et al.* (Canadian Patent Application 2,125,240 A1), and further in view of Yelton *et al.* (The Journal of Immunology 155:1994-2002 [1995]) and Hagiwara *et al.* (US Patent No. 5,589,573, issued 12/1996). Applicants cannot agree. There is no proper basis for the combination of the cited art. Moreover, even if the art is (improperly) combined, all of the elements of the presently pending claims are not taught.

A. There Is No Basis For The Combination

To establish *prima facie* obviousness, the Examiner must point to some motivation or suggestion within the references themselves, or within the knowledge generally available to one of ordinary skill in the art at the time of invention, to combine or modify the references. *See* MPEP §2143.01; *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988). Merely because the references ***could be*** combined or modified does not render the resultant combination obvious unless the prior art suggested the combination. MPEP §2143.01; *In re Mills*, 916 F.2d 680, 682, 16 USPQ2d 1430, 1432 (Fed. Cir. 1990).

Applicants submit that the references cannot be considered collectively until the Examiner points to ***some motivation to combine*** those references. The purpose behind this requirement is to prevent the Examiner from using the invention itself and hindsight reconstruction to defeat the patentability of the invention. The Federal Circuit, in a recent decision, articulates this position:

> To prevent the use of hindsight based on the invention to defeat patentability of the invention, this court requires the examiner to show a motivation to combine the references that create the case of obviousness. In other words, the examiner must show reasons that the skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed.

See In re Rouffet et al., 149 F.3d 1350, 47 USPQ2d 1453 (Fed. Cir. 1998). It is readily apparent that the law of *In re Rouffet* requires the Examiner to present soundly reasoned arguments based upon the substance of the cited references.[2] Moreover, the law does not regard the Examiner as one skilled in the art. *See In re Rijckaert*, 28 USPQ2d 1955 at 1956 (Fed. Cir. 1993)("[T]he examiner's assumptions do not constitute the disclosure of the prior art."); *See id.* at 1957 ("[W]hen the PTO asserts that there is an explicit or implicit teaching or suggestion in the prior art, it must indicate where such a teaching or suggestion appears."). Indeed, the Federal Circuit has made it clear that "[b]road, conclusory statements regarding the teachings of multiple references, standing alone, are not 'evidence.'" *In re Dembiczak*, 175 F.3d 994, 999, 50 USPQ2d 1614 (Fed. Cir. 1999).

Applicants submit that the Examiner has not provided a sound explanation for combining these references as required by the law in *In re Rouffet*. What the Examiner has provided are unsupported and conclusory statements. In this regard, a review of Hagiwara reveals that it is not an antibody engineering reference, merely an antibody cloning reference with no apparent teaching to make changes in the sequence. Similarly, the Yelton reference only involves CDR changes and does not utilize the overlapping oligo approach as presently claimed. Why would one skilled in the art combine such disparate art and techniques? The Examiner is reminded that there are many techniques in use

B. Even If (Improperly) Combined, All Elements Are Not Taught

Applicants remind the Examiner that the presently pending claims, which represent but one embodiment of the invention, are directed to simultaneous modification of a donor CDR and an acceptor framework region (i.e. each member of the resulting population has a change in each region),[3] wherein the acceptor framework is modified based on comparison to a particular donor framework region reference sequence. While Examiner takes the position that Deng *et al.* teach mutagenesis in both the CDRs and the frameworks (page 6, third paragraph, Office Action mailed 5/13/02), Applicants are unable to find the specific teaching

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[2] *Accord Ex parte Clapp*, 227 USPQ 972 (Bd. Pat. App. & Inter. 1985) (stating that the examiner must present convincing line of reasoning supporting rejection).

[3] The claims have been amended at step (d) to specifically emphasize this feature of the resulting population (of this embodiment of the invention). The amendment was made to further the prosecution and without waiving the right to prosecute the unamended claims (or similar claims) in the future.

in Deng *et al.* for CDR and framework region modifications on the same molecule.

i. Deng Does Not Teach Simultaneous CDR and FR Changes

As Applicants noted in the previous office action response (mailed 3/22/02), none of the examples in Deng *et al.* appear to be directed to the synthesis of antibodies with the simultaneous modification of CDR and FR residues. Specifically, Example 1 is directed to a one-stage affinity maturation of an antibody by simultaneous modification of three CDRs. The example does not discuss framework modifications. Indeed, as noted in the previous response, the figure with the sequencing data from this example (Figure 4) has a figure description (page 6, lines 16-18 of Deng *et al.*) which notes that "Some deletions (indicated by D) and framework mutations, **thought to be DNA synthesizer errors**, were also observed." (emphasis added). As already argued (page 14 of the response mailed 3/22/02), introduction of random errors during oligonucleotide synthesis is clearly NOT the same as the introduction of a modification (a different or changed amino acid) at one or more framework regions, wherein said framework positions that are changed are selected from among those which differ between a reference acceptor sequence and the corresponding positions of a reference donor sequence.[4] Furthermore, in this affinity maturation example of Deng *et al.* (Example 1), the framework regions appear to be unchanged during the affinity maturation process [*i.e.* the framework regions of the donor and the acceptor appear to be identical, from the same antibody, as the affinity maturation process described by Deng *et al.* appears to involve replacing CDRs in a given antibody framework with modified CDRs in the same antibody framework (barring DNA synthesizer errors)]. Note that Table 2 (page 18 of Deng *et al.*) refers to the table as "CDR randomized Library".

Turning to Example 2 of Deng *et al.*, this example describes a two-stage in vitro affinity maturation of a single chain antibody. Again, according to the text of Deng *et al.* (page 20, line 29- page 22, line 29), the in vitro affinity maturation libraries are "CDR-randomized". There is a heavy chain CDR-randomized library and a light chain CDR-randomized library. On page 21 (lines 22-24), the two sequences that emerged are described as having a single CDR2 mutation (one sequence) and two CDR3 mutations (the other sequence). Again, Applicants fail to find the teaching for simultaneous CDR and

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[4] Thus, it is not enough for the Examiner to merely point to some random error as a framework change - since the claims require that the changes be of a certain type.

framework modifications in this Example. And, as the Example is directed to affinity maturation, the inference is that the same framework is being used.

Turning now to Example 3 of Deng *et al.*, which is directed to a humanization library of randomized framework residues. In this example, the murine Sel55-4 VH is used as a source of CDR sequences, and the human antibody NEW is used as a source of framework region sequences, to replace murine Sel55-4 VH framework regions 1-3. In this case, the example teaches framework modifications *in the absence of CDR modifications* (the example does not mention modifying any of the CDRs in any of the oligos).

Thus, NONE of the three examples presented in Deng *et al.* present simultaneous CDR and FR modifications, wherein the FR modifications are based on comparisons between a specific donor reference sequence and a specific acceptor reference sequence.

Applicants note that the Examiner has cited three portions of Deng *et al.* to support the characterization that Deng *et al.* allegedly teach that the CDRs are randomized and the framework regions are randomized (see page 4, third paragraph of the office action mailed 5/13/02). These three portions have been earnestly and diligently reviewed - but a teaching of **simultaneous** CDR and FR modifications is not to be found. The Examiner cites page 12, lines 28-32 of Deng *et al.*, which, upon review, refers to the humanization of rodent antibodies, and the randomization of selected framework residues in human frameworks in an example. There is no suggestion to simultaneously modify framework and CDR residues. The Examiner also cites Figure 2 of Deng *et al.* . Figure 2 shows the construction scheme to accompany Example 1 (affinity maturation), and as noted in the description of Figure 2 (page 5, lines 28-35 of Deng *et al.*), the figure shows the simultaneous randomization of three CDRs. The double arrows in Figure 2 are said to show the five, eight and six amino acid stretches (in CDR1, CDR2 and CDR3, respectively) that were mutated by the spiking procedure. Applicants respectfully submit that Figure 2 of Deng *et al.* fails to describe simultaneous CDR and FR modification, wherein the FR residues that are altered are selected from among those which differ between a reference acceptor sequence and the corresponding positions of a reference donor sequence. Finally, the Examiner cites pages 22-23 of Deng *et al.* These pages correspond to the Tables of Example 2 and the description of Example 3 of Deng *et al.* As already noted (*supra*), Applicants find that Example 2 is directed to CDR modifications in affinity maturation while Example 3 is directed to FR modifications in

humanization, and respectfully submit that neither are directed to simultaneous CDR and FR modifications, wherein the FR residues that are altered are selected from among those which differ between a reference acceptor sequence and the corresponding positions of a reference donor sequence.

ii. Deng Teaches Away

Interestingly, the abstract of Deng *et al.* apparently teaches away from the simultaneous modification of CDR and FR residues, as it states that the method can be used for randomizing antibody complementarity determining regions **or** framework regions (emphasis added). This is reiterated on page one of Deng *et al.* (lines 11-15), where the invention is said to be exemplified with methods for randomizing antibody complementarity determining regions **or** framework regions (emphasis added).

iii. The Use of A Polymerase - As Claimed - Is Not Taught

Turning now to other claim elements which the Examiner alleges are taught by Deng *et al.*, specifically, the use of a polymerase as specified in claims 57 and 62. As claimed in Claims 57 and 62, the embodiments in which a polymerase is specified use the polymerase in step d) to extend the overlapping oligonucleotides created in step c). The Examiner characterizes Deng *et al.* as teaching PCR of the DNA and as teaching that overlapping oligonucleotides can be used in the method which eliminate the template DNA (see page 7, last sentence of the office action mailed 5/13/02).

Deng *et al.* has been carefully studied for use of polymerases, and the use of a polymerase to extend overlapping oligonucleotides is not to be found. Rather, Deng *et al.* consistently teach and use the ligase chain reaction or standard T4 ligations to join the oligonucleotides into a construct (see for example page 3, line 35- page 4, line 8). In some cases, oligonucleotides are joined in the presence of template DNA, while in some cases template DNA may be eliminated, which may result in a more stringent requirement for overlapping oligonucleotide ends (see page 4, lines 1-3 of Deng *et al.*). Nevertheless, as described by Deng *et al.*, the joining of the oligonucleotides is carried out in a ligation reaction, using a ligase (and NOT a polymerase).

The Examiner notes (page 7, last sentence of the Office Action mailed 5/13/02) that Deng *et al.* teach PCR of the DNA. Applicants respectfully point out that Deng *et al.* appear to use PCR only on ligase products (*i.e.* on products of the ligase reaction, which have the

oligonucleotides already covalently joined by ligase). For example, on page 4, lines 14-16 of Deng *et al.*, it is noted that the "mutated full-length ligase products are then directly cloned into a display vector or amplified by PCR (polymerase chain reaction)." This is clearly NOT the same as using a polymerase to extend overlapping oligonucleotides to construct a population of altered variable region encoding nucleic acids. That is, the use of a polymerase in the presently claimed embodiment is to synthesize a construct from ovelapping oligonucleotides. Deng *et al.* use a polymerase (in some cases) to amplify a ligase product, in which oligonucleotides have been joined by ligase, and using PCR primers which are NOT the same as the oligonucleotides comprising the construct (see, for example, PCR primers P1 and P2 in the middle panel of Figure 2 of Deng *et al.*). Figure 2 of Deng *et al.* clearly shows ligase chain reaction carried out on oligonucleotides, agarose gel isolation of this product and only then PCR amplification, using a different primer set for the amplification. Even in Example 3 of Deng *et al.*, which appears to be the only example in which ligase reactions are carried out in the absence of template DNA (in one reaction), the ligase reaction is carried out in the absence of a polymerase. PCR is only used after completion of the ligase reaction.

Applicants thus respectfully submit that the use of PCR (and a polymerase) by Deng *et al.* is not taught as presently claimed. Thus Deng *et al.* fails to render the use of a polymerase to extend overlapping oligonucleotides obvious (particularly when said overlapping populations of oligonucleotides comprise CDR and FR modifications, and when said FR residues that are changed are selected based on comparison between a donor and acceptor reference sequence).

iv. The Other References Do Not Remedy The Deficiencies

The Examiner has already admitted that Deng *et al.* fails to teach electronic reference sequences and fails to teach the use of codon-based mutagenesis (page 5 of the Office Action mailed 5/13/02). Applicants respectfully submit that Deng *et al.* also fail to teach (a) the simultaneous modification of CDR and FR residues, wherein the FR residues that are modified are selected based on comparison between a donor and acceptor reference sequence and (b) the use of a polymerase to extend overlapping oligonucleotides such that a population of altered variable region encoding nucleic acids is constructed. Applicants respectfully submit that neither Yelton *et al.* nor Hagiwara *et al.* make up for all of these deficiencies in Deng *et al.*

Specifically, Yelton *et al.* is directed to affinity maturation of a heavy chain variable region. Yelton *et al.* use the method of Kunkel *et al.* for the mutagenesis strategy, which does not involve the use of populations of overlapping oligonucleotides. While Yelton *et al.* use codon-based mutagenesis, Yelton *et al.* teach affinity maturation, which (as described by Yelton *et al.*) involves the construction of CDR libraries to be placed in the same antibody framework (*i.e.* the CDRs of antibody BR96 are subjected to codon-based mutagenesis, and returned to the BR96 framework). Thus, the strategy is for CDR randomization only.

Turning to Hagiwara *et al.*, while the sequence analysis (particularly the analysis of the hypervariable regions) does involves electronic database searching, Hagiwara *et al.* fail to make up the above-noted deficiencies of Deng et al. While Hagiwara teaches amplification of antibody genes, cloning and sequencing, there is no apparent teaching regarding antibody engineering so as to change the sequences. Certainly, there is no teaching of overlapping populations of overlapping oligos with simultaneous CDR and FR modifications, wherein the FR residues that are changed are selected based on comparing the sequences of two reference sequences. Thus, Hagiwara adds nothing to the Deng reference.

CONCLUSION

Applicants believe that the arguments set forth above traverse the Examiner's rejections and therefore request that these grounds for rejection be withdrawn for the reasons set forth above. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicants encourage the Examiner to call the undersigned collect at (617)-252-3353.

Dated: September 13, 2002

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APPENDIX I
MARKED-UP VERSION OF AMENDED CLAIMS

Please amend the claims as shown below:

42. (Twice Amended) A method of constructing a population of altered heavy chain variable region encoding nucleic acids, comprising:

a) providing [a] visual representations of first and second reference amino acid sequences, said first reference amino acid sequence comprising the sequence of a donor heavy chain variable region, said donor variable region comprising i) donor framework regions and ii) three donor complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference amino acid sequence comprising the sequence of an acceptor heavy chain variable region comprising acceptor framework regions;

b) synthesizing i) a first population of oligonucleotides, comprising oligonucleotides encoding a modified heavy chain variable region framework region, or portion thereof, wherein said modified heavy chain variable region framework region, or portion thereof, contains a plurality of changed amino acids at one or more positions when compared to said acceptor framework regions of said second reference sequence, wherein said framework positions of said modified heavy chain variable region that are changed are selected from among said acceptor framework positions of said second reference sequence that differ at the corresponding position compared to the donor framework positions of said first reference sequence; and ii) a second population of oligonucleotides, each encoding at least one modified complementarity-determining region, or portion thereof, wherein said modified complementarity-determining region, or portion thereof, comprises a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region amino acid reference sequence, and one or more portions of adjacent framework regions which are capable to hybridizing to said first population of oligonucleotides; and

c) mixing said first and second populations of oligonucleotides so as to create overlapping oligonucleotides; and

d) treating said overlapping oligonucleotides under conditions such that a population of altered heavy chain variable region encoding nucleic acids is constructed,

wherein each altered heavy chain variable region encoding nucleic acid of said population of altered heavy chain variable region encoding nucleic acids encodes for a polypeptide, said polypeptide comprising i) a different amino acid at one or more positions when compared to said corresponding acceptor framework regions of said second reference sequence, and ii) a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region of said first reference sequence.

43. (Amended) The method of Claim 42, wherein said visual representations of first and second reference sequences [is] are in electronic form.

47. (Twice Amended) A method of constructing a population of altered light chain variable region encoding nucleic acids, comprising:

a) providing [a] visual representations of first and second reference amino acid sequences, said first reference amino acid sequence comprising the sequence of a donor light chain variable region, said donor variable region comprising i) donor framework regions and ii) three donor complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference amino acid sequence comprising the sequence of an acceptor light chain variable region comprising acceptor framework regions;

b) synthesizing i) a first population of oligonucleotides, comprising oligonucleotides encoding a modified light chain variable region framework region, or portion thereof, wherein said modified light chain variable region framework region, or portion thereof, contains a plurality of changed amino acids at one or more positions when compared to said acceptor framework regions of said second reference sequence, wherein said framework positions of said modified light chain variable region that are changed are selected from among said acceptor framework positions of said second reference sequence that differ at the corresponding position compared to the donor framework positions of said first reference sequence; and ii) a second population of oligonucleotides, each encoding at least one modified complementarity-determining region, or portion thereof, wherein said modified complementarity-determining region, or portion thereof, comprises a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region amino acid reference sequence and one or more portions of adjacent

framework regions which are capable of hybridizing to said first population of oligonucleotides; and

c) mixing said first and second populations of oligonucleotides so as to create overlapping oligonucleotides; and

d) treating said overlapping oligonucleotides under conditions such that a population of altered light chain variable region encoding nucleic acids is constructed, wherein each altered light chain variable region encoding nucleic acid of said population of altered light chain variable region encoding nucleic acids encodes for a polypeptide, said polypeptide comprising i) a different amino acid at one or more positions when compared to said corresponding acceptor framework regions of said second reference sequence, and ii) a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region of said first reference sequence.

48. (Amended) The method of Claim 47, wherein said visual representations of first and second reference sequences [is] are in electronic form.

52. (Amended) A method of constructing a population of altered heavy chain variable region encoding nucleic acids, comprising:

a) providing [a] visual representations of first and second reference amino acid sequences, said first reference amino acid sequence comprising the sequence of a donor heavy chain variable region, said donor variable region comprising i) donor framework regions and ii) three donor complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference amino acid sequence comprising the sequence of an acceptor heavy chain variable region comprising acceptor framework regions;

b) synthesizing i) a first population of oligonucleotides, comprising oligonucleotides encoding a modified heavy chain variable region framework region, or portion thereof, wherein said modified heavy chain variable region framework region, or portion thereof, contains a plurality of changed amino acids at one or more positions when compared to said acceptor framework regions of said second reference sequence, wherein said framework positions of said modified heavy chain variable region that are changed are selected from among said acceptor framework positions of said second reference sequence that

differ at the corresponding position compared to the donor framework positions of said first reference sequence; and ii) a second population of oligonucleotides, each encoding at least one modified complementarity-determining region, or portion thereof, wherein said modified complementarity-determining region, or portion thereof, comprises a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region amino acid reference sequence and one or more portions of adjacent framework regions which are capable of hybridizing to said first population of oligonucleotides; and

c) mixing said first and second populations of oligonucleotides so as to create overlapping oligonucleotides; and

d) extending said overlapping oligonucleotides with a DNA polymerase under conditions such that a population of altered heavy chain variable region encoding nucleic acids is constructed, wherein each altered heavy chain variable region encoding nucleic acid of said population of altered heavy chain variable region encoding nucleic acids encodes for a polypeptide, said polypeptide comprising i) a different amino acid at one or more positions when compared to said corresponding acceptor framework regions of said second reference sequence, and ii) a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region of said first reference sequence.

53. (Amended) The method of Claim 52, wherein said visual representations of first and second reference sequences [is] are in electronic form.

57. (Amended) A method of constructing a population of altered light chain variable region encoding nucleic acids, comprising:

a) providing [a] visual representations of first and second reference amino acid sequences, said first reference amino acid sequence comprising the sequence of a donor light chain variable region, said donor variable region comprising i) donor framework regions and ii) three donor complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference amino acid sequence comprising the sequence of an acceptor light chain variable region comprising acceptor framework regions;

b) synthesizing i) a first population of oligonucleotides, comprising

oligonucleotides encoding a modified light chain variable region framework region, or portion thereof, wherein said modified light chain variable region framework region, or portion thereof, contains a plurality of changed amino acids at one or more positions when compared to said acceptor framework regions of said second reference sequence, wherein said framework positions of said modified light chain variable region that are changed are selected from among said acceptor framework positions of said second reference sequence that differ at the corresponding position compared to the donor framework positions of said first reference sequence; and ii) a second population of oligonucleotides, each encoding at least one modified complementarity-determining region, or portion thereof, wherein said modified complementarity-determining region, or portion thereof, comprises a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region amino acid reference sequence and one or more portions of adjacent framework regions which are capable of hybridizing to said first population of oligonucleotides; and

c) mixing said first and second populations of oligonucleotides so as to create overlapping oligonucleotides; and

d) extending said overlapping oligonucleotides with a DNA polymerase under conditions such that a population of altered light chain variable region encoding nucleic acids is constructed, wherein each altered light chain variable region encoding nucleic acid of said population of altered light chain variable region encoding nucleic acids encodes for a polypeptide, said polypeptide comprising i) a different amino acid at one or more positions when compared to said corresponding acceptor framework regions of said second reference sequence, and ii) a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region of said first reference sequence.

58. (Amended) The method of Claim 57, wherein said visual representations of first and second reference sequences [is] are in electronic form.

62. (Amended) A method of constructing a population of altered heavy chain variable region encoding nucleic acids, comprising:

a) providing [a] visual representations of first and second reference amino acid

sequences, said first reference amino acid sequence comprising the sequence of a donor heavy chain variable region, said donor variable region comprising i) donor framework regions and ii) three donor complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference amino acid sequence comprising the sequence of an acceptor heavy chain variable region comprising acceptor framework regions;

b) synthesizing i) a first population of oligonucleotides, comprising oligonucleotides encoding a modified heavy chain variable region framework region, or portion thereof, wherein said modified heavy chain variable region framework region, or portion thereof, contains a plurality of changed amino acids at one or more positions when compared to said acceptor framework regions of said second reference sequence, wherein said framework positions of said modified heavy chain variable region that are changed are selected from among said acceptor framework positions of said second reference sequence that differ at the corresponding position compared to the donor framework positions of said first reference sequence, and wherein said changed amino acids were introduced through the use of codon-based mutagenesis; and ii) a second population of oligonucleotides, each encoding at least one modified complementarity-determining region, or portion thereof, wherein said modified complementarity-determining region, or portion thereof, comprises a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region amino acid reference sequence, and wherein said different amino acid was introduced through the use of codon-based mutagenesis and one or more portions of adjacent framework regions which are capable of hybridizing to said first population of oligonucleotides; and

c) mixing said first and second populations of oligonucleotides so as to create overlapping oligonucleotides; and

d) treating said overlapping oligonucleotides under conditions such that a population of altered heavy chain variable region encoding nucleic acids is constructed, wherein each altered heavy chain variable region encoding nucleic acid of said population of altered heavy chain variable region encoding nucleic acids encodes for a polypeptide, said polypeptide comprising i) a different amino acid at one or more positions when compared to said corresponding acceptor framework regions of said second reference sequence, and ii) a different amino acid at one or more positions when compared to the corresponding donor

complementarity-determining region of said first reference sequence.

63. (Amended) The method of Claim 62, wherein said visual representations of first and second reference sequences [is] are in electronic form.

67. (Amended) A method of constructing a population of altered light chain variable region encoding nucleic acids, comprising:

a) providing [a] visual representations of first and second reference amino acid sequences, said first reference amino acid sequence comprising the sequence of a donor light chain variable region, said donor variable region comprising i) donor framework regions and ii) three donor complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference amino acid sequence comprising the sequence of an acceptor light chain variable region comprising acceptor framework regions;

b) synthesizing i) a first population of oligonucleotides, comprising oligonucleotides encoding a modified light chain variable region framework region, or portion thereof, wherein said modified light chain variable region framework region, or portion thereof, contains a plurality of changed amino acids at one or more positions when compared to said acceptor framework regions of said second reference sequence, wherein said framework positions of said modified light chain variable region that are changed are selected from among said acceptor framework positions of said second reference sequence that differ at the corresponding position compared to the donor framework positions of said first reference sequence, and wherein said changed amino acids were introduced through the use of codon-based mutagenesis; and ii) a second population of oligonucleotides, each encoding at least one modified complementarity-determining region, or portion thereof, wherein said modified complementarity-determining region, or portion thereof, comprises a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region amino acid reference sequence, and wherein said different amino acid was introduced through the use of codon-based mutagenesis and one or more portions of adjacent framework regions which are capable of hybridizing to said first population of oligonucleotides; and

c) mixing said first and second populations of oligonucleotides so as to create

overlapping oligonucleotides; and

d) treating said overlapping oligonucleotides under conditions such that a population of altered light chain variable region encoding nucleic acids is constructed, wherein each altered light chain variable region encoding nucleic acid of said population of altered light chain variable region encoding nucleic acids encodes for a polypeptide, said polypeptide comprising i) a different amino acid at one or more positions when compared to said corresponding acceptor framework regions of said second reference sequence, and ii) a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region of said first reference sequence.

68. (Amended) The method of Claim 67, wherein said visual representations of first and second reference sequences [is] are in electronic form.